Development and Validation of Stability-indicating HPTLC Method for Simultaneous estimation of Montelukast Sodium and Bilastine as Bulk Drugs and in Combined Tablet Formulation

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ABSTRACT

The present work describes the development and validation of a new simple, accurate, precise and selective stability-indicating high-performance thin layer chromatographic (HPTLC) method for simultaneous determination of Montelukast sodium and Bilastine in combined tablet dosage form. The chromatographic separation was carried out using precoated silica gel $60F_{254}$ (10 × 10 cm) plates as stationary phase and a mixture of Toluene: Ethyl acetate: Methanol (7: 1: 2, v/v/v) as mobile phase. Retention factors for Montelukast sodium and Bilastine were found to be 0.62±0.02 and 0.26±0.02, respectively. The wavelength selected for detection was 280 nm. Drug samples were subjected to different stress conditions like acidic, alkaline, hydrolysis, oxidation, photolysis, and thermal degradation. The developed method has been validated for linearity, accuracy, precision, limit of detection, and limit of quantification and robustness, as per ICH guidelines. Results were found to be linear in the concentration range of 500-3000 ng $band^{-1}$ for Montelukast and 1000-6000 ng $band^{-1}$ for Bilastine, respectively. The percentage drug contents (Mean ± S.D.) obtained for Montelukast Sodium and Bilastine were 99.37 ± 1.65 and 99.95 ± 0.97, respectively. The developed method can be used for the simultaneous quantification of these drugs in combined tablet dosage form as well as for routine analysis in quality control laboratories.

Keywords: Bilastine, Montelukast sodium, HPTLC, Stability Studies, Validation

INTRODUCTION

Montelukast, sodium, chemically, 2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl) ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl methyl] cyclopropyl] acetate is used in the maintenance treatment of asthma [1]. Bilastine, chemically, 2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropanoic acid is an antihistamine drug used to treat urticaria, allergic rhinitis and itchy inflamed eyes (allergic conjunctivitis) caused by an allergy [2].

An extensive literature review revealed that analytical methods such as UV spectrophotometry [3], High-Performance Liquid Chromatography (HPLC) [4-6] have been reported for the determination of Montelukast in pharmaceutical formulations either as single or in combination with other drugs. Analytical methods such as UV spectrophotometry [7], and HPLC [8, 9] were also available for estimation of Bilastine in pharmaceutical formulations either as single or in combination with other drugs. Analytical methods such as UV spectrophotometry [10], RP-HPLC [11-13] and HPTLC [14] were also reported in the literature for simultaneous estimation of Montelukast and Bilastine.

To best of our knowledge, no reports were available in the literature for simultaneous determination of Montelukast sodium and Bilastine in the combined tablet dosage form by stability indicating HPTLC method. Based on this observation, we have developed a selective, accurate, precise high-performance thin layer chromatography method for the simultaneous estimation of Montelukast and Bilastine in combined tablet dosage form in accordance with the International Conference on Harmonisation Guidelines [15, 16].

MATERIALS AND METHODS

Chemicals and reagents

Working standards Bilastine and Montelukast Sodium were obtained from Zuventus Healthcare Ltd. (Pune, India). The pharmaceutical formulation Bilamove M tablets containing 10 mg Montelukast sodium and 20 mg Bilastine (Glenmark Pharmaceuticals Ltd. India) were procured from the local pharmacy. Toluene and Methanol (AR grade) were obtained from Thomas Baker Pvt Ltd (Mumbai, India). Ethyl acetate was obtained from Loba Chemie Pvt Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drugs was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 5 mm width using Camag 100 µL sample syringe

(Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: ethyl acetate: methanol (7: 1: 2, v/v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner III at 280 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Selection of analytical wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that both drugs showed considerable absorbance at 280 nm. So, 280 nm was selected as the wavelength for detection.

Stock solution and working standard preparation

Accurately weighed 10 mg of Montelukast was dissolved in 10 mL of methanol to get the solution having concentration 1000 µg mL^{-1} which was diluted further with methanol to acquire a final working concentration of 250 ng μL^{-1}. The standard solution for Bilastine was prepared by dissolving accurately weighed 20 mg in 10 mL of methanol to get the solution having concentration 2000 µg mL^{-1} from which 2.5 mL of solution was diluted with same solvent to get solution having final concentration 500 ng μL^{-1}

Assay of marketed formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 10 mg of Montelukast (20 mg Bilastine) was weighed and transferred to 10 mL volumetric flask containing 7 mL of methanol. The contents were sonicated for 20 min, filtered and volume was made with methanol. From this stock solution, 2.5 mL was further diluted to 10 mL with methanol. 4 µL volume of this solution was applied on TLC plate to obtain final concentration of 1000 ng $band^{-1}$ for Montelukast and 2000 ng $band^{-1}$ for Bilastine. After chromatographic development peak areas of the bands were measured at 280 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies

The stability studies were performed by subjecting the standard solution of bulk drugs to physical stress (hydrolysis, peroxide, heat, and light) and stability was accessed. The stress degradation studies were carried out at an initial drug concentration of 1000 µg mL^{-1} of Montelukast and 2000 µg mL^{-1} of Bilastine in methanol. The hydrolytic studies were carried

out by mixing the drug solutions of Montelukast and Bialstine separately with 1 N HCl and 1 N NaOH. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 250 ng band^{-1} and 500 ng band^{-1} of Montelukast and Bilastine, respectively. The oxidative degradation was carried out in 6 % H_2O_2 and the sample was diluted with methanol to obtain a solution having concentration 250 ng band^{-1} and 500 ng band^{-1} of Montelukast and Bilastine, respectively. Thermal stress degradation was performed by keeping the solid drugs individually in oven at 50ºC for a period of 2 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square meter^{-1}. Thermal and photolytic samples were diluted with methanol to get the concentration of 250 ng band^{-1}and 500 ng band^{-1} of Montelukast and Bilastine, respectively.

RESULTS AND DISCUSSION

Method optimization

The main aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drugs from each other and also from their degradation products. Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally the mobile phase comprising toluene: ethyl acetate: methanol (7: 2: 1, v/v/v) was selected as optimal for obtaining well-defined and resolved peaks for the drugs. A densitometric evaluation was carried out at 280 nm. The retention factors were found to be 0.62±0.02 and 0.26±0.02 for Montelukast and Bilastine, respectively. A representative densitogram of a mixed standard solution of both drugs is shown in Figure 1.

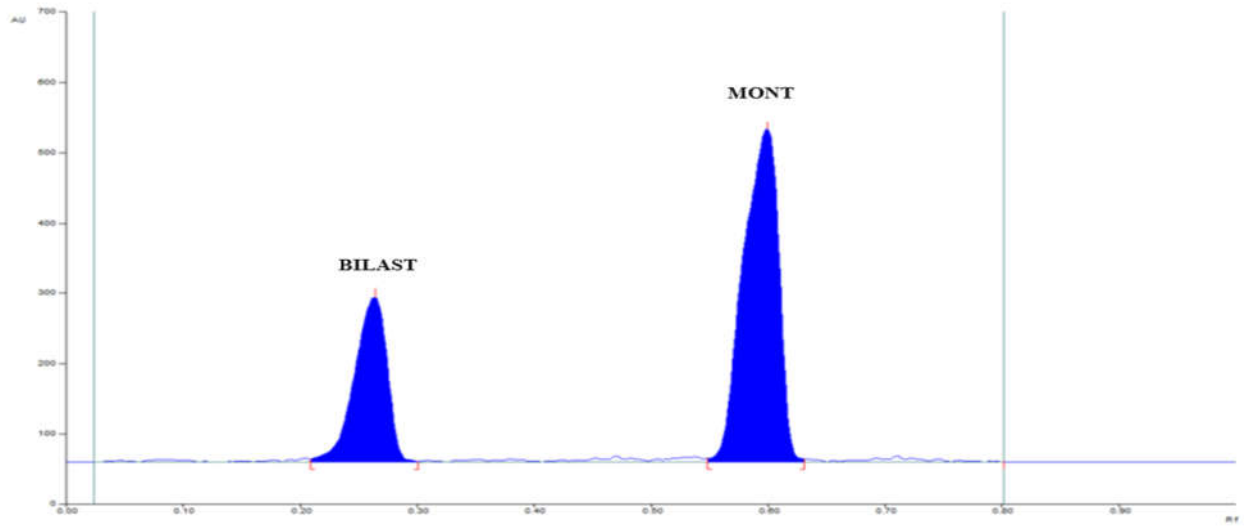


Figure 1: Representative Densitogram of Bialstine (4000 ng/band, Rf = 0.26±0.05) and Montelukast (2000 ng/band, Rf = 0.62±0.05)

3.2 Forced degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative, thermal stress conditions and stability under photolytic stress conditions. Marked degradation in the densitograms was observed with the appearance of degradation products for Bilastine under acid hydrolysis and peroxide treatment. Significant degradation also found for Montelukast without appearance of any peak for degradation products. The degradation products formed were not interfering with active drug which indicated specificity of developed method. Figures 2 and 3 shows the densitograms of acid and peroxide degradation. The unaffected assay of tablet formulation confirmed the stability indicating the power of the method. The findings of degradation studies are represented in Table 1.

Table 1: Stress degradation studies

Stress conditions/ duration	Montelukast		Bilastine	
	% Recovery	% degradation	% Recovery	% degradation
Acidic / 1N HCl	82.26	17.74	76.79	23.21
Alkaline /1 N NaOH	88.72	11.28	82.21	17.79
Oxidative /6 % H_2O_2	86.18	13.82	79.43	20.57
Dry heat/ 50ºC/ 2 h	85.03	14.97	91.18	8.81
Photolysis	99.81	-----	99.37	-----

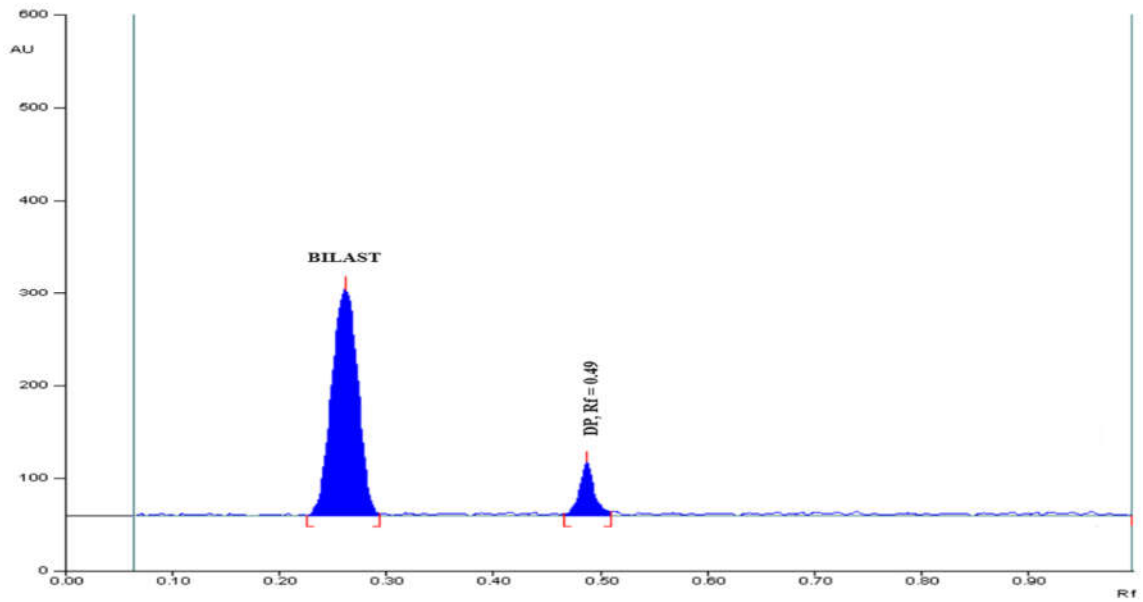


Figure 2: Densitogram of Bilastine obtained after acid degradation with degradation product (DP1, Rf = 0.49)

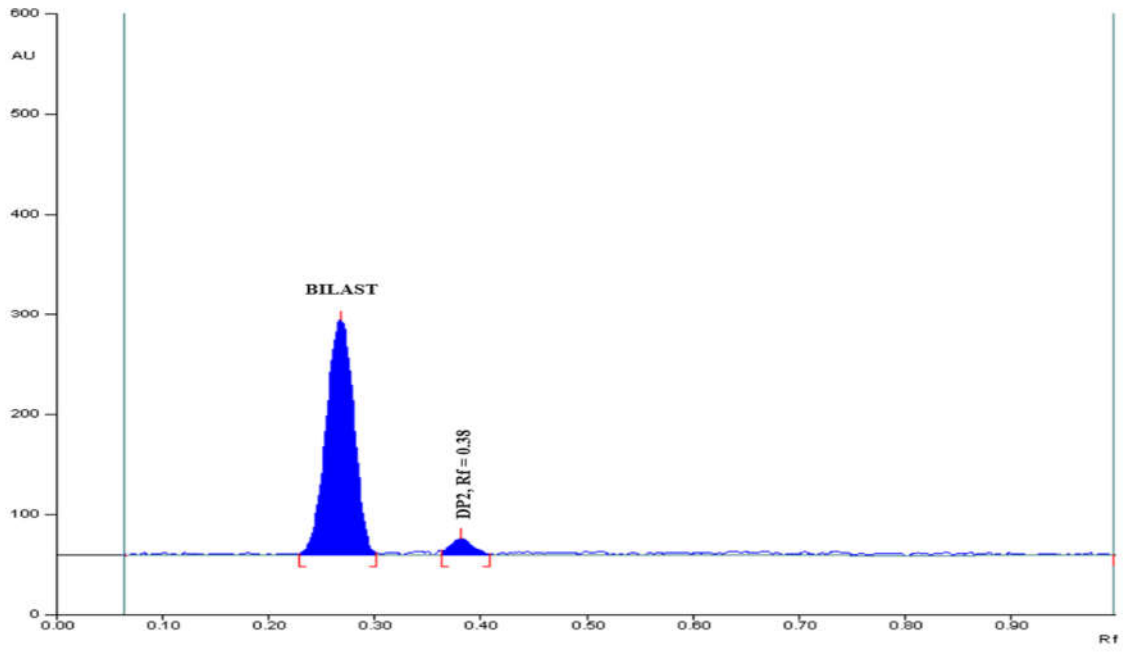


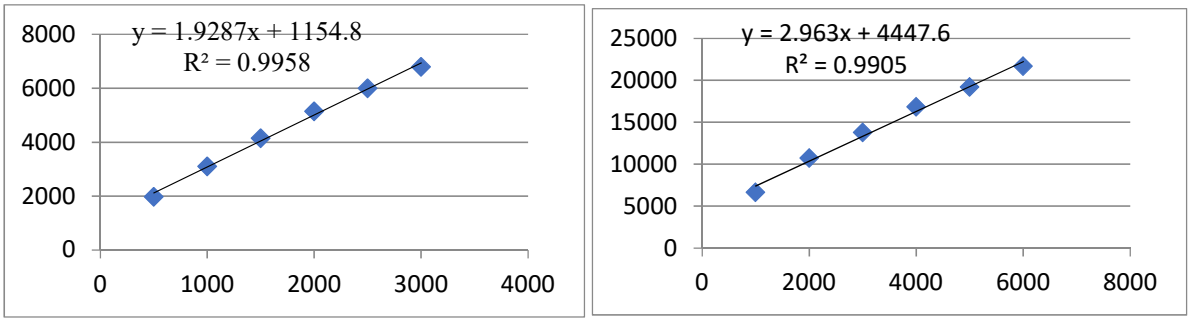
Figure 3: Densitogram of Bilastine obtained after peroxide treatment with degradation product (DP2, Rf = 0.38)

Analytical method validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness.

Linearity and Range

Volumes 2, 4, 6, 8, 10 and 12 µL of the standard stock solutions of Montelukast and Bilastine were applied by over spotting on TLC plate to obtain the concentration in the range 500-3000 ng band^{-1} for Montelukast and 1000-6000 ng band^{-1} for Bilastine, respectively. Results were found to be linear in the concentration range indicated above. The linear regression equation and correlation coefficient were found to be y = 1.9287x + 1154.8 and R^2 = 0.995 for Montelukast and y = 2.963x + 4447.6 and R^2 = 0.990 for Bilastine, respectively.



(a) **(b)**

Figure 4: Calibration curve for (a) Montelukast (b) Bilastine

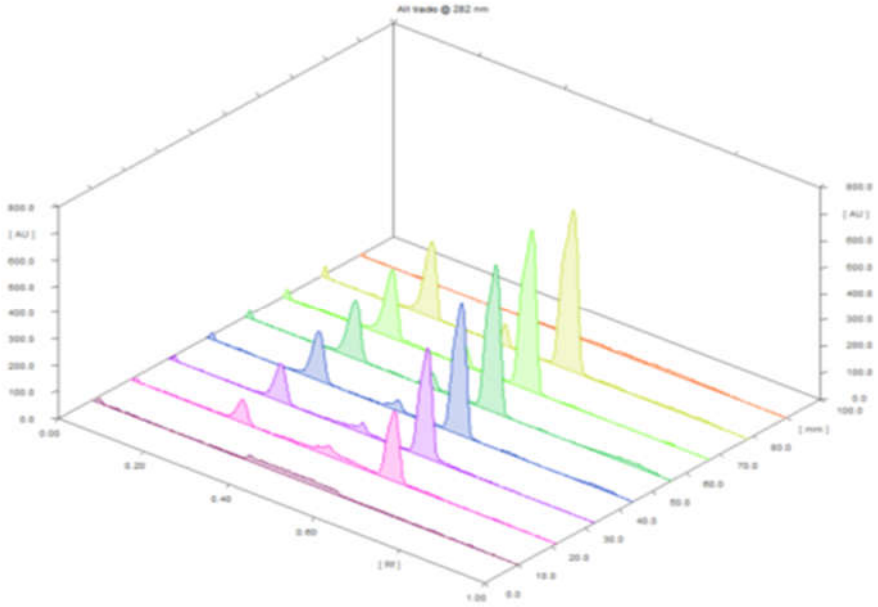


Figure 5: 3D spectra of linearity for Bilastine (1000-6000 ng band^{-1}) and Montelukast (500-3000 ng band^{-1})

Precision

Set of three different concentrations in three replicates of mixed standard solutions of Montelukast Sodium and Bilastine were prepared and were analyzed on the same day and on three consecutive days. Intra-day variation, as RSD (%), was found to be in the range of 0.66 to 1.25 for Montelukast Sodium and 0.84 to 1.29 for Bilastine. Interday variation, as RSD (%) was found to be in the range of 0.92 to 1.70 for Montelukast Sodium and 0.78 to 1.56 for Bilastine.The smaller values of % R.S.D. obtained indicate that the developed method is precise.

Table 2: Intraday precision studies

Drug	Spotted Conc. (ng band^{-1})	Conc. Found (ng band^{-1})	% Recovery	% R.S.D.*
Bilastine	3000	3012.50	100.41	1.29
	4000	4011.83	100.29	0.84
	5000	5009.58	100.18	1.15
Montelukast Sodium	1500	1505.78	100.38	1.25
	2000	1991.08	99.55	1.13
	2500	2483.29	99.32	0.66

*Average of three determinations

Table 3: Inter-day precision

Drug	Spotted Conc. (ng band^{-1})	Conc. Found (ng band^{-1})	% Recovery	% R.S.D.*
Bilastine	3000	2984.15	99.46	1.56
	4000	4002.49	100.05	0.93
	5000	4992.14	99.83	0.79
Montelukast Sodium	1500	1491.60	99.43	1.56
	2000	2005.24	100.25	1.70
	2500	2494.52	99.77	0.92

*Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD values of Montelukast Sodium and Bilastine were found 150.1 ng band^{-1} and 298.78 ng band^{-1} and, respectively. The LOQ values of Montelukast Sodium and Bilastine were 454.84 ng band^{-1} and 905.41 ng band^{-1}, respectively.

Accuracy

Recovery studies were carried out to check accuracy of the method by standard addition method. It involved addition of standard drug to pre-analysed sample at three different levels 80, 100 and 120 %. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drugs in combined tablet dosage form

Table 4: Recovery studies

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total Amount of the drug(ng/band)	Amount found (ng band^{-1})	% Recovery±R.S.D.*
Bilastine	2000	1600	3600	3595.45	99.87
	2000	2000	4000	3976.39	99.40
	2000	2400	4400	4368.34	99.27
Montelukast Sodium	1000	800	1800	1789.23	100.45
	1000	1000	2000	2006.34	100.31
	1000	1200	2200	2192.77	99.68

*Average of three determinations, R.S.D. is relative standard deviation

Robustness

Robustness of the method was determined by making deliberate variations in method parameters. The parameters like mobile phase composition (± 1% methanol), wavelength (± 1 nm) were altered and the effect on the area of drugs was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

CONCLUSION

Stability indicating HPTLC method was developed for simultaneous estimation of Montelukast sodium-Bilastine combination. The developed method is simple, precise, selective, and accurate. Stability indicating the nature of the method was confirmed by performing stress degradation of drugs under different conditions viz. hydrolysis, oxidation, thermal, and photolysis. Both Montelukast and Bilastine were found to be susceptible to acid and base-catalyzed hydrolysis, oxidation, and thermal stress conditions. Both drugs showed stability under photolytic stress conditions. The peaks obtained for degradation products did not merge with drug peaks which denote the specificity of the developed method. The proposed method may be used for routine analysis of these drugs in quality control laboratories and can also be beneficial for monitoring the potency of this combination during shelf life.

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